Prevention by Estrogen of the Decidual Alkaline Phosphatase Response in Intact and Ovariectomized Pseudopregnant Rats (34620)

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In intact rats, or in rats castrated on day 4 and receiving progesterone replacement, the decidual alkaline phosphatase activity was 12 times greater on day 8 of pseudopregnancy than in the contralateral nontraumatized horn (1–3). This pseudopregnant decidual enzyme response was entirely similar to the alkaline phosphatase reaction in day 8 implantation sites of pregnant rats (1, 3–6). No increased enzyme was elicited by the nondecidualized uteri of nontraumatized, pseudopregnant animals treated with 17β-estradiol and/or progesterone or rats ovariectomized and traumatized on day 4 and given only estrogen. Additionally, the changes in alkaline phosphatase from day 5 to 10 in the intact and hormone-treated ovariectomized pseudopregnant rats with traumatized uteri were comparable to those demonstrated by the intact pregnant animals, *i.e.*, an increased reaction from day 5 to 8 and decreased activity from day 8 to 10 (1). These data supported the view that the requirements for the elevation of decidual alkaline phosphatase in the pseudopregnant animal were similar to those in the pregnant rat; that is, physical and/or chemical irritation (day 4) and a progesterone-sensitized endometrium (1, 3, 5, 6). The results for the pseudopregnant deciduae, however, do not entirely exclude the possibility of some residual ovarian estrogen entering directly into the mechanism of alkaline phosphatase stimulation because the traumatized animals were ovariectomized and treated with hormones starting on day 4 of pseudopregnancy.

The purpose of this study was to discover whether estrogen is necessary for the rise in decidual alkaline phosphatase activity and, if so, when and how much is required. In order to exlude the influence of ovarian estrogen, rats castrated on day 1 of pseudopregnancy were utilized for this investigation.

Materials and Methods. The rats used were 180–200 g adult virgin albinos (KG Farms). The animals were housed in an air-conditioned, controlled light-reverse room (12 hr of light, 12 hr of darkness). Standard rat chow and water were available *ad libitum*. Only animals with normal 5-day estrus cycles by vaginal smear were used.

Pseudopregnancy was induced between 10–12 AM by electric stimulation (constant voltage of 9 V) for 30 sec of the cervix uteri at proestrus and estrus. This time was designated as day 0 of pseudopregnancy. Bilateral ovariectomy and/or unilateral uterine trauma (multiple pinching of one uterine horn from the oviduct to approximately 1 cm above the cervix) was performed under ether anesthesia between 10–12 AM respectively, on day 1 and 4. The 17β-estradiol (General Biochemicals, 0.1 or 1.0 μg/0.25 ml sesame oil) and/or progesterone (USP, 5 mg/0.25 ml sesame oil) were injected subcutaneously. See Table I for the exact treatment schedule.

The animals were killed by decapitation on the eighth day following the electric cervical stimulation. Not all traumatized animals had a decidual reaction; however, the tissues from traumatized and nontraumatized uteri of rats with and without deciduae were assayed. The uterine horns were removed, weighed on an analytical balance, and transferred to an ice-cooled ground glass homogenizer containing cold distilled water. The tissue was minced at a high speed (1000 rpm) until a uniform suspension was obtained (usually 3–4 min). Alkaline phosphatase determinations were done on 0.1% whole homogenates using a dual channel AutoAnalyzer. The technique and manifold are essentially

TABLE I. Data for Control and Test Rats Concerning Uterine Alkaline Phosphatase Response.[a]

Group no.	Day number						Animals			Alkaline phosphatase activity (units[b]/g dry wt, mean ± SE)			
				17β-Estradiol						Decidualized		Nondecidualized	
	ECS	UT	OX	0.1 μg/day	1.0 μg/day	Proges. 5 mg/day	Total	Dec.	Nondec.	TH	NTH	TH	NTH
1	0	—	—	—	—	—	10	0	10	—	—	—	46 ± 4
2	0	4	—	—	—	—	10	8	2	620 ± 44	34 ± 2	58 ±17	77 ±30
3	0	4	—	—	1–7	1–7	10	0	10	—	—	29 ± 2	29 ± 2
4	0	4	—	—	1–7	—	10	1	9	457	44	48 ± 5	52 ± 7
5	0	4	—	1–3	4–7	—	10	5	5	574 ±117	31 ± 3	41 ± 8	51 ±11
6	0	4	—	1–4	5–7	—	28	10	18	487 ± 75	34 ± 7	35 ± 4	47 ± 6
7	0	4	—	1–3	4–7	1–7	19	10	9	616 ± 76	29 ± 2	22 ± 3	27 ± 3
8	0	4	—	—	—	1–7	10	8	2	730 ± 63	50 ± 5	32 ± 1	38 ± 6
9	0	4	1	—	1–7	1–7	10	1	9	600	34	29 ± 2	33 ± 1
10	0	4	1	—	1–7	—	6	0	6	—	—	55 ± 6	68 ± 7
11	0	4	1	1–3	4–7		10	0	10	—	—	36 ± 3	54 ± 4
12	0	4	1	1–3	4–7	1–7	9	7	1	578 ± 56	42 ± 9	37 ± 3	38 ± 1
13	0	4	1	—	3	1–7	10	9	1	608 ± 52	60 ± 4	37	45
14	0	4	1	—	4	1–7	10	8	2	616 ± 81	57 ± 2	58 ±13	57 ± 2
15	0	4	1	—	5	1–7	10	9	1	516 ± 45	28 ± 4	16	19
16	0	4	1	—	—	1–7	8	7	1	601 ± 83	56 ± 4	61	42

[a] ECS = Electrical cervical trauma
UT = Uterine trauma
OX = Ovariectomy
TH = Traumatized horn
NTH = Nontraumatized horn

[b] 1 unit of alkaline phosphatase activity liberates 1 mg phenol from 40 m*M* phenylphosphate at pH 10.5 and 37° in 15 min under standard conditions.

the same as that described by Fishman and Green, (7) except that the sample tubes were increased from a 0.015 to 0.020 in. i.d. to facilitate delivery of the homogenate. The optimal substrate concentration (disodium phenylphosphate, 40m*M*) and pH (10.5) were previously determined (8).

Results. The data are presented in Table I. The untraumatized, nondecidualized, control animals had a uterine alkaline phosphatase reaction of 46 units/g dry weight. Decidualized uteri of untreated intact rats with unilateral uterine trauma had an increased enzyme activity at 620 units on day 8 of pseudopregnancy. This enhanced alkaline phosphatase response was not altered in decidualized uteri of either intact animals treated with seven daily injections of 5 mg of progesterone and/or 17β-estradiol at 0.1 μg on days 1 to 3 and 1.0 μg on days 4 to 7, or in ovariectomized rats receiving the progestin alone on days 1 to 7 or combined with the estrogen as above or given a single 1.0 μg dose of 17β-estradiol on days 3, 4, or 5. Extending the low-dose estrogen treatment to day 4 was without effect in the intact rat. However, no decidualization or increased enzyme reaction was found in intact or castrated animals receiving 1.0 μg of 17β-estradiol with or without 5 mg of progesterone for days 1 to 7. Similarly, ovariectomized rats treated with only the 0.1 μg dose of estrogen on days 1 to 3 with 1.0 μg on days 4 to 7 also failed to show decidualization and increased enzyme activity.

Enzyme activity of nondecidualized tissue from traumatized, or contralateral untraumatized, uterine horns, whether from treated or untreated rats, was comparable to that found in controls whose uteri were not physically stimulated. Intact rats treated with 0.1 μg of estrogen on days 1 to 3 or 4 and with 1.0 μg on days 4 or 5 to 7 showed respectively 50 and 35% decidualization compared with the 80% rate recorded for untreated traumatized controls. Only 53% of the intact animals receiving the combination of progesterone and 17β-estradiol at 0.1 μg on days 1 to 3 and 1.0 μg on days 4 to 7 were decidualized.

Discussion. As previously reported, intact rats with traumatized and decidualized uteri demonstrated a 10–15-fold increase in alkaline phosphatase on day 8 of pseudopregnancy (1–3). Enzyme concentrations of the nondecidualized contralateral horns remained similar to the nontraumatized pseudopregnant animals. High levels of histochemical alkaline phosphatase have been described in deciduae after traumatization of the endometrium in the pseudopregnant rat and mouse (9, 10). The present data further substantiate our original postulate (1, 3, 5, 6) that the rise in decidual alkaline phosphatase was dependent upon physical and/or chemical irritation of a progesterone conditioned endometrium. Thus, the decidual enzyme reaction was not affected by treating (a) intact animals with seven daily injections of 5 mg progesterone and/or 17β-estradiol, 0.1 μg on days 1–3 and 1.0 μg on days 4–7, and (b) ovariectomized rats with progesterone alone or combined with either the 0.1 μg on days 1–3, 1.0 μg on days 4–7, estrogen regimen, or a single 1.0-μg dose of 17β-estradiol on days 3, 4, or 5. If 1.0 μg of 17β-estradiol was administered with or without 5 mg progesterone for days 1–7, however, no decidualization or rise in alkaline phosphatase was noted in intact or castrated animals. Increased enzyme levels also failed to occur in the nondecidualized ovariectomized rats receiving the 0.1 μg on days 1–3, 1.0 μg on days 4–7, estrogen regimen, due to a lack of progesterone replacement therapy. The observations clearly demonstrate that the enhanced alkaline phosphatase activity was independent of 17β-estradiol treatment as long as the estrogen did not prevent decidualization.

The low level of 17β-estradiol used in this study may be near threshold for the prevention of decidualization, because the number of intact rats receiving estrogen at a dose of 0.1 μg on days 1–3 or 4 and 1 μg on days 4 or 5–7 demonstrating deciduae was respectively, 50 and 35% compared to the 80% response rate of the traumatized intact controls. Additionally, concomitant administration of progesterone will not inhibit this effect since rats treated with 17β-estradiol, 0.1 μg

on days 1–3, 1.0 μg days 4–7, and progesterone, 5 mg on days 1–7, showed only a 53% decidualization rate. The enzyme reaction of the individual deciduae was not altered.

In earlier studies on intact animals, decidualization, implantation, and increased alkaline phosphatase activity could be prevented by administration of 1.0 μg of 17β-estradiol on days 1–7 of pregnancy (3–5). A single 10 μg injection of estradiol cyclopentylpropionate on day 1 was observed by Greenwald (11) to inhibit ova implantation in pregnant rats by accelerating egg transport in the oviduct. Since implantation has never been shown (to our knowledge) to occur in the absence of decidualization in the rat, and, in view of the present results with pseudopregnant animals, we propose that a blastocyst in the uterus at the optimal nidation time (between day 4 and 5) in the estrogen-treated pregnant rat (1.0 μg and greater, depending on the compound) would not have implanted due to a lack of a developing decidua.

Summary. The decidual alkaline phosphatase of traumatized intact pseudopregnant rats was 10–15 times greater on day 8 than nondecidualized uteri. This effect was not altered by treatment of (a) intact rats with 5 mg of progesterone and/or 17β-estradiol, 0.1 μg on days 1–3, 1.0 μg on days 4–7, daily, and (b) castrated animals with the progestin (days 1–7) alone or combined with either the above estrogen regimen, or a single 1.01 μg dose of 17β-estradiol on days 3, 4, or 5. If 1.0 μg of 17β-estradiol was administered with or without progesterone for days 1–7, no decidualization or rise in alkaline phosphatase was noted. Similarly, the enzyme failed to increase in nondecidualized castrated rats receiving the 0.1 μg on days 1–3, 1.0 μg on days 4–7, estrogen regimen. The data demonstrate that the decidual alkaline phosphatase response is independent of circulating ovarian estrogen.

Estrogen (0.1 μg on days 1–3 or 4, 1.0 μg on days 4 or 5–7) appeared to reduce the number of intact rats exhibiting decidualization and enhanced enzyme activity.

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